

다중의약품에 저항하는 *Staphylococcus aureus* 균에 항균성을 가지는 파라-히드록시벤조히드라자이드 유도체의 합성과 구조-활성관계 3차원 정량분석

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Synthesis and 3D-QSAR of *p*-Hydroxybenzohydrazide Derivatives With Antimicrobial Activity Against Multidrug-Resistant *Staphylococcus aureus*

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요약. 40여년전에 보고된 이래 병원에서 유래한 메치실린-저항 *Staphylococcus aureus* (MRSA) 은 세계적으로 큰 문제가 되어왔다. 항균성의 가능성을 가지는 새로운 약품을 개발하기 위하여 N²-[(-3-substituted-4-oxo-1,3-thiazolidin-2-ylidene)-4-hydroxy benzohydrazide (**4a-4.i**)]와 N²-[(-3,4-disubstituted)-1,3-thiazolidin-2-ylidene]-4-hydroxybenzohydrazide (**5.a-5.i**) ~ (**10.a-10.i**)을 적절한 합성방법을 사용하여 합성하였다. 이들 합성된 화합물들은 *s. aureus* 균주에 대해 생체의 조건에서 분석하였다. 치료 화합물과 표준 화합물에 대해 최소억제농도(MIC)를 결정하였다. 시험한 모든 화합물들은 2000 µg/mL 투여량까지는 독성이 없었고, 사용한 균주에 대해 상당한 항균성을 보였다. 특히 **6.f**, **7.g**, **9.f**와 **10.f**, **10.i**들이 가장 항균성이 컸다. 이것으로 미루어 파라-히드록시벤조히드라자이드 고리와 치환된 싸이아졸린 고리는 항균성에 필수적임을 알 수 있었다. 3D-QSAR 분석결과로 파라-히드록시벤조히드라자이드의 활성자리에 대한 결합방식을 알게되었다.

주제어: 구조-활성관계 3차원 정량분석, 상, 항균성, 파라-히드록시벤조히드라자이드

ABSTRACT. Hospital-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) has been an increasing problem worldwide since the initial reports over 40 years ago. To examine new drug leads with potential antibacterial activities, Various N²-[(-3-substituted-4-oxo-1,3-thiazolidin-2-ylidene)-4-hydroxy benzohydrazide (**4a-4.i**)] and N²-[(-3,4-disubstituted)-1,3-thiazolidin-2-ylidene]-4-hydroxybenzohydrazide from (**5.a-5.i**) to (**10.a-10.i**) were synthesized using appropriate synthetic route. The entire test compounds (**4.a-4.i**) and from (**5.a-5.i**) to (**10.a-10.i**) were assayed *in vitro* against *s. aureus* strain. The minimum inhibitory concentration (MIC) was determined for test compounds and for reference standards. The test compounds showed significant antibacterial activity against the strains used, when tested *in vitro*. In general, *p*-hydroxybenzohydrazide ring and substituted thiazoline ring are essential for antimicrobial activity. Among the compounds tested, compounds **6.f**, **7.g**, **9.f** and **10.f**, **10.i** were found to be most potent. The test compounds were found nontoxic upto the dose level of 2000 µg/mL. The intact compounds were then subjected for 3D-QSAR studies. 3D-QSAR study based on the principal of alignment of pharmacophoric features by Schrödinger PHASE module. The 3D-QSAR study allowed us to confirm the preferential binding mode of *p*-hydroxybenzohydrazide inside the active site.

Keywords: 3D-QSAR, PHASE, Antimicrobial, *p*-Hydroxybenzohydrazide

INTRODUCTION

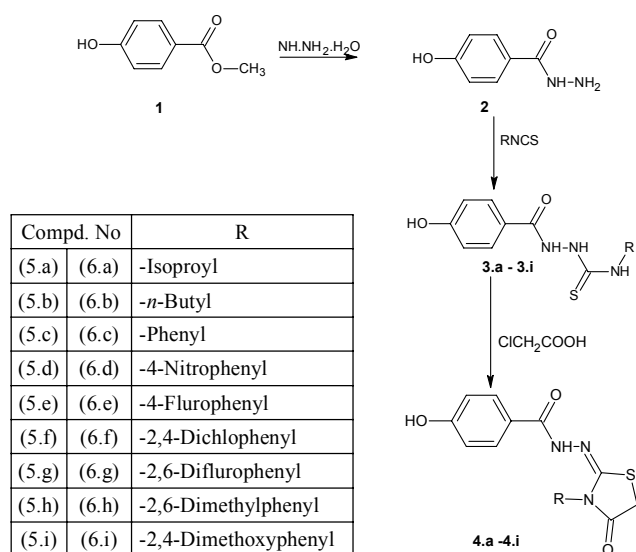
The deterioration of human population due to the enhance prevalence of infectious diseases is becoming a worldwide problem. Over the last few years, The resistance of bacteria against antimicrobial agents has become a widespread medical problem especially as nosocomial pathogens. Treatment options for these infections are often limited, especially in debilitated

and immunocompromised patients.^{1,2} In the last decade, there has been a reemergence of Gram-positive bacteria, in particular *Staphylococcus aureus*, which is considered one of the main causes of nosocomial infections.³⁻⁵ The infectious disease caused by MRSA (methicillin-resistant *Staphylococcus aureus*) is currently a serious problem because these bacteria show a multidrug-resistant phenotype, that is, resistance not only to methicillin but also to several other drugs except vancomycin

and teicoplanin. Although potent antistaphylococcal drugs are available, this infection continues to present significant morbidity and mortality rates, justifying the need for the development of more effective compounds for its treatment.⁶⁻⁹ properties allows the medicinal chemist to identify which features are important or not for biological activity resulting, in a way, in a successful lead development process. With the constant advancement of QSAR (quantitative structure-activity relationships) studies as a molecular modification approach, this procedure has been applied in several scientific areas.

In order to use these medicinal chemistry advances to counter the high incidence of antibiotic-resistant microorganisms, this study is aimed at the design, synthesis, and determination of antimicrobial activity of N⁷-[(3-Sub-4-oxo-1,3-thiazolidin-2-ylidene)-4-hydroxy benzohydrazide (**4a-4.i**) and N⁷-[(3,4 Disubstituted)-1,3-thiazolidin-2-ylidene)]-4-hydroxybenzohydrazide **from (5.a-5.i) to (10.a-10.i)** against *s. aureus* strains.^{10,11}

Discovering three-dimensional pharmacophores which can explain the activity of a series of ligands is one of the most significant contributions of computational chemistry to drug discovery.¹² Quantitative drug design embraces two major activities, the quantitative description of the structural differences among series of chemical compounds of biological interest, and the formulation of "QSAR" useful in the design of new and better therapeutic agents.¹³ It should also be possible to make connections from such activity models to structure-based design, either to add more information to overlays for the construction of a pharmacophore model or to use a pharmacophore to assist in the refinement of protein homology models.¹⁴ In the present model QSAR model has been developed for the prediction of *S. aureus*. Inhibition. 3-D QSAR approach had been developed using PHASE module of Schrödinger suite.¹⁴



Scheme 1

CHEMISTRY

The synthesis of the intermediate and target compounds were performed by the reaction illustrated in *Scheme 1* and *Scheme 2*.

Compound 2 namely 4-hydroxybenzohydrazide was synthesized in excellent yield by amination of hydrazine hydrate. Reaction of 2 with alkyl/aryl isothiocyanate in ethanol gives compounds (**3.a-3.i**). The structures of the compounds **3.a-3.i** were confirmed on the basis of elemental analysis and spectral data. The IR spectra showed NH and CS stretching bands at 3215 - 3230 and 1309 - 1348 cm⁻¹, respectively. The ¹H NMR showed downfield signal at δ 11.6 - 14.23 attributed to 3-substituted NH. The reaction of **3.i-3.i** with chloroacetic acid in boiling ethanol containing fused sodium acetate afforded the corresponding N⁷-[(2Z)-3-(4-Substituted alkyl/aryl thiazolidin-2-ylidene)]-4-hydroxy benzohydrazide. In fact, only one product was obtained as confirmed by TLC. The structure of the products (**4.a-4.i**) was based on previous discussion of the structures of similar compounds.¹⁵ So the compounds-{(2Z)-2-[(4-alkyl/aryl-substituted)imino]-4-oxo-1,3-thiazolidin-3-yl}-2-(4-hydroxybenzo)acetamide as expected from our previous discussion will not formed. Condensation of product (**3.a-3.i**) with 4-substituted phenacyl bromides affords compounds **from (5.a-5.i) to (10.a-10.i)**. The structure of the products **from (5.a-5.i) to (10.a-10.i)** was based on previous discussion of the structures of similar compounds.²¹ The structures of the reaction products were confirmed by elemental analysis, IR, ¹H NMR and FABMS analyses. IR spectra revealed that the disappearance of NH band at 3215 - 3230 cm⁻¹.

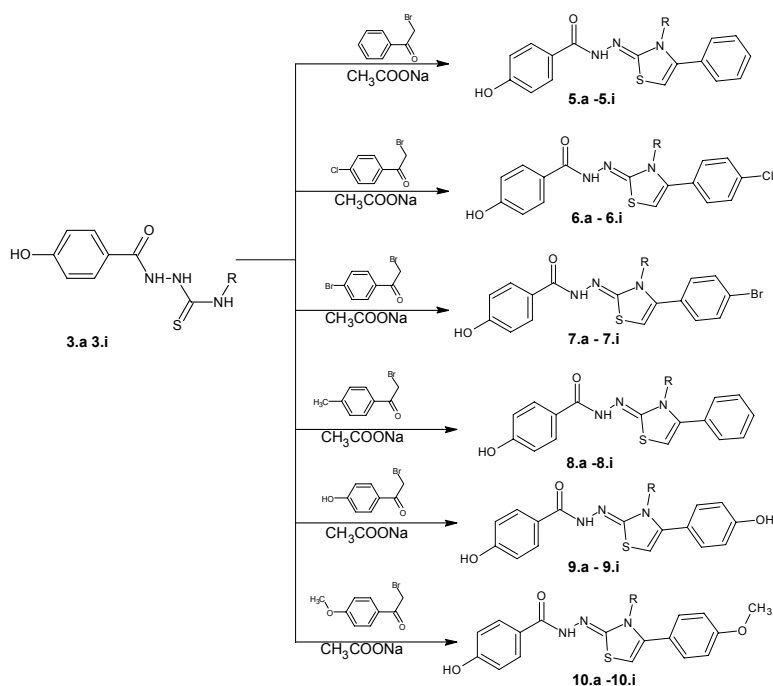
The ¹H NMR spectra also lacked the NH signals and showed new singlet signal at δ 5.8 - 6.1 attributed to C₅-H of thiazoline ring.

Antimicrobial activity

Compounds **4.a-4.i** and **from (5.a-5.i) to (10.a-10.i)** *in vitro* against species of Gram-positive bacteria, *staphylococcus aureus* (ATCC 3750). The MIC values were also tested for standard drugs like Nifuroxazide, Chloramphenicol were used as a standard.

3D-QSAR Study of synthesized compounds

We performed 3D-QSAR analysis on the series N⁷-[(3-Sub-4-oxo-1,3-thiazolidin-2-ylidene)]-4-hydroxy benzohydrazide **4.a-4.i** and N⁷-[(3,4-Disubstituted)-1,3-thiazolidin-2-ylidene)]-4-hydroxybenzohydrazide **from (5.a-5.i) to (10.a-10.i)** were synthesized using appropriate synthetic route. The entire test compounds **4.a-4.i** and compounds **from (5.a-5.i) to (10.a-10.i)**. The software use for 3D-QSAR study is Schrödinger PHASE¹⁴ Module.



Scheme 2

Compd. No						R
5.a	6.a	7.a	8.a	9.a	10.a	Isoproyl
5.b	6.b	7.b	8.b	9.b	10.b	<i>n</i> -Butyl
5.c	6.c	7.c	8.c	9.c	10.c	Phenyl
5.d	6.d	7.d	8.d	9.d	10.d	4-Nitrophenyl
5.e	6.e	7.e	8.e	9.e	10.e	4-Fluorophenyl
5.f	6.f	7.f	8.f	9.f	10.f	2,4-Dichlophenyl
5.g	6.g	7.g	8.g	9.g	10.g	2,6-Difluorophenyl
5.h	6.h	7.h	8.h	9.h	10.h	2,6-Dimethylphenyl
5.i	6.i	7.i	8.i	9.i	10.i	2,4-Dimethoxyphenyl

RESULTS AND DISCUSSION

N^2 -[(-3-Sub-4-oxo-1,3-thiazolidin-2-ylidene)-4-hydroxy benzohydrazide (**4.a-4.i**) and N^2 -[(3,4-Disubstituted)-1,3-thiazolidin-2ylidene]-4-hydroxy benzohydrazide from (**5.a-5.i**) to (**10.a-10.i**) were synthesized and evaluated for their physical, analytical and spectral data. This selectivity in the scheme is believed to be due to electron density at N_2 and N_1 . The former being richer in electron density, is more reactive and produces products of exclusive functionalization at N_2 .

Antibacterial activity

The MIC values of the test compounds are summarized in Table 3. For comparison, the MICs of compounds **2**, and **3.a-3.i** are included in Table 1. The results revealed that the test compounds under Scheme 2 exhibit remarkable antimicrobial activity against *S.aureus* strain. The MIC values are in the range of 4 - 69 $\mu\text{g}/\text{mL}$. For structure activity studies we choose the aromatic substitutions that are commonly employed in *p*-hydroxybenzohydrazide. Thiazoline ring is essential for antibacterial activity as compounds **2**, **3.a-3.i** showed comparatively less activity than from (**5.a-5.i**) to (**10.a-10.i**). Amide linkage and thiazoline ring contributes significantly towards antimicrobial activity. The different substituent in compounds from (**5.a-5.i**) to (**10.a-10.i**) over the side chain at 3 and 4 position of thiazoline ring exerts significant influence on biological activity. In general, aromatic substituted compounds at 3-position were found to be more active than aliphatic substituents. Further, the pre-

sence of electron-withdrawing groups (both halogen and nitro substituents) in **5.a-5.i** showed maximum antimycobacterial activity. Literature survey reveals that electrons-withdrawing or donating groups amend the lipophilicity of the test compounds, which in turn alters permeability across the bacterial cell membrane. Again in comparison to thiazoline ring, thiazolidine ring showed less activity **4.a-4.i**. Thus thiazolidine ring is not essential for imparting the antibacterial activity to the compounds containing 4-hydroxybenzohydrazide ring. Further, the toxicity of most potent compounds (**6.f**, **7.g**, **9.f** and **10.f**, **10.i**) were assessed using LD_{50} values, their corresponding LD_{50} values were found to be within the range of 560 - 770 $\mu\text{g}/\text{mL}$ (**6.f**, 750; **7.g**, 630; **9.f**, 570; **10.f**, 670; **10.i**, 590 $\mu\text{g}/\text{mL}$). Thus, in comparison with drugs commonly used in therapy, our most potent compounds showed similar or slightly less *in vitro* antimicrobial activity against *s.aureus* with marked reduction in toxicity (hemolytic activity), suggested that this class of compounds could be used as potent broadspectrum antimicrobial agent to treat various clinical conditions associated with multiple infectious diseases. Further studies are in progress to optimize these lead compounds and to characterize the mode of action.

3D-QSAR study

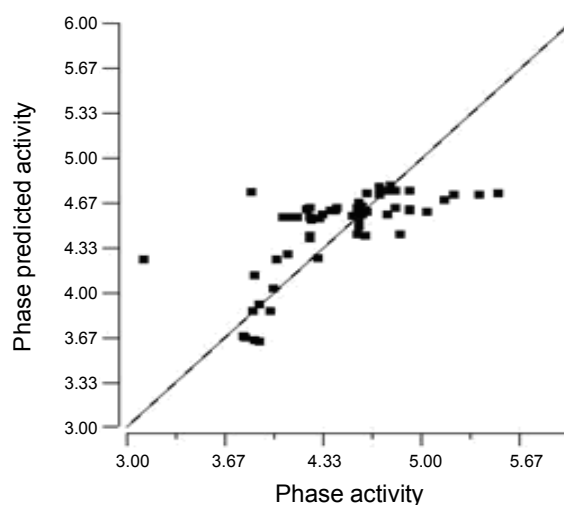
The 3D-QSAR studies for the set of benzohydrazide and their derivatives were carried out using PHASE module of Schrödinger molecular modeling package. For finding the common pharmacophore hypothesis, the dataset was divided into active and inactive sets. Molecules with pIC_{50} values more than 4.50

Table 1. *In-vitro* antimicrobial activity

Compd. No.	Antibacterial activity ($\mu\text{g/mL}$) ^{a,b}	Compd. No.	Antibacterial activity ($\mu\text{g/mL}$) ^{a,b}
(3.a)	56	(6.f)	27
(3.b)	49	(6.g)	15
(3.c)	56	(6.h)	16
(3.d)	59	(6.i)	15
(3.e)	38	(7.a)	27
(3.f)	23	(7.b)	59
(3.g)	32	(7.c)	56
(3.h)	32	(7.d)	79
(3.i)	25	(7.e)	29
(4.a)	24	(7.f)	07
(4.b)	26	(7.g)	17
(4.c)	95	(7.h)	12
(4.d)	26	(7.i)	26
(4.e)	55	(8.a)	57
(4.f)	06	(8.b)	79
(4.g)	16	(8.c)	46
(4.h)	14	(8.d)	26
(4.i)	13	(8.e)	23
(5.a)	14	(8.f)	09
(5.b)	26	(8.g)	19
(5.c)	85	(8.h)	37
(5.d)	25	(8.i)	04
(5.e)	25	(9.a)	56
(5.f)	41	(9.b)	49
(5.g)	3	(9.c)	56
(5.h)	19	(9.d)	59
(5.i)	25	(9.e)	38
(6.a)	56	(9.e)	23
(6.b)	56	(9.g)	32
(6.c)	69	(9.h)	32
(6.d)	26	(9.i)	25
(6.e)	17	(10.a)	24
(10.b)	26	(10.f)	06
(10.c)	95	(10.g)	16
(10.d)	26	(10.h)	14
(10.e)	55	(10.i)	13
Std ²	3.1	Std ¹	3.5

^aDMF has no antimicrobial activity at the concentration used to dissolve the test compounds. ^bMIC: minimum inhibitory concentration.

were considered to be active, and those with pIC50 values less than 4.10 (Table 2, Fig. 1) were considered to be inactive, whereas those in-between were considered to be moderately active. A common pharmacophore model ADHRRR (Table 3, Fig. 2a) with two variants was generated after the creation and identification of pharmacophoric sites in all the molecules in the dataset. The variant with a site score 0.98, vector score 0.91, and volume score of 0.80 (Table 4) was chosen to be the common pharmacophore hypothesis. The pharmacophore hypothesis ADHRRR with all active molecules aligned to it is shown in Fig. 2. All the molecules in the active set/modelled

Fig. 1. Phase predicted vs phase activity (pIC₅₀) for 3D-QSAR study.

molecules matched with the hypothesis ADHRRR. This pharmacophore hypothesis was then used for the generation of QSAR model. For the QSAR model generation, non modeled (inactive or moderately active) molecules in the dataset were then aligned based on the matching with at least three of the pharmacophoric features. The dataset was randomly divided into a training set of 51 compounds and 12 in the test set with a bias given to the structural diversity in both the training and test set so as to form the standard 4:1 training set to test set ratio for a QSAR study.

The PHASE statistical analysis for each of the test set selection methods is summarized in Table 3. The validity of each of the models was predicted from the calculated correlation coefficient for the randomly chosen test set comprising of diverse structures. The squared correlation for the test set (random selection ($R^2_{\text{pred}} = 0.91$)) confirms the good predictability of the final QSAR model for the test set.

Analysis of Atom-Based PHASE 3D-QSAR Model: Fig. 2 shows the volume occlusion maps for the atom-based PHASE 3D-QSAR model (donor, hydrophobic, and electronegative) represented by black and gray codes. These maps represent the regions of favorable and unfavorable interactions. The volume occlusion maps of hydrogen bond donor (Fig. 2c) describe the spatial arrangement of favorable hydrogen bonding interactions to acceptor groups of the target protein. The volume occlusion maps of electron-withdrawing groups (Fig. 2b) indicate the suitable position of electron-withdrawal groups in the thiazole rings of the benzohydrazide. These analyses indicate that improvements in the receptor binding affinity can be achieved by substituting electron-withdrawing groups on the thiazole ring of benzohydrazide moieties at 3rd positions. Positions 4/5 in the thiazole ring or phenyl ring is not favorable for the electron-withdrawing groups. Hydrophobic volume occlusion maps from

Table 2. Data set used for 3-D QSAR analysis with corresponding actual and predicted pIC₅₀ activity of compds as *S. aureus* inhibitor

Compd. No.	<i>S. aureus</i> inhibitor			Compd. No.	<i>S. aureus</i> inhibitor		
	Actual pIC ₅₀	Predicted pIC ₅₀	Residuals		Actual pIC ₅₀	Predicted pIC ₅₀	Residuals
(4.a)	4.31	4.39	0.08	(7.f)*	4.40	4.56	0.26
(4.b)	3.839	3.93	0.10	(7.g)*	5.96	5.17	-0.79
(4.c)	3.757	4.06	0.31	(7.h)	4.85	4.84	-0.01
(4.d)	3.999	3.82	0.17	(7.i)	4.25	4.49	0.24
(4.e)	4	3.85	-0.15	(8.a)	4.46	4.42	-0.04
(4.f)	3.951	3.84	-0.11	(8.b)	4.32	4.50	0.18
(4.g)*	3.988	4.08	0.10	(8.c)	4.12	4.55	0.43
(4.h)	4.111	4.12	0.01	(8.d)	4.32	4.49	0.17
(4.i)	3.728	3.97	0.25	(8.e)	4.55	4.46	-0.09
(5.a)*	4.19	4.40	0.21	(8.f)	5.22	4.56	-0.66
(5.b)	4.44	4.47	0.03	(8.g)	4.72	4.67	-0.05
(5.c)	4.31	4.53	0.22	(8.h)	4.62	4.66	0.04
(5.d)	4.23	4.48	0.25	(8.i)	4.87	4.69	-0.18
(5.e)	3.98	3.93	-0.05	(9.a)*	4.37	4.42	0.05
(5.f)	4.23	4.51	0.28	(9.b)	4.79	4.64	-0.15
(5.g)*	4.79	4.65	0.14	(9.c)	5.12	4.66	-0.46
(5.h)	4.60	4.55	-0.05	(9.d)*	4.14	4.31	0.17
(5.i)	5.24	4.82	-0.42	(9.e)	4.74	4.54	-0.20
(6.a)	4.02	4.42	0.40	(9.f)	4.72	4.68	-0.04
(6.b)*	4.79	4.51	-0.28	(9.g)	4.88	4.96	0.08
(6.c)*	4.60	4.54	-0.06	(9.h)	4.40	4.65	0.25
(6.d)	4.79	4.52	-0.27	(9.i)	4.16	4.56	0.40
(6.e)	3.99	4.54	0.55	(10.a)	4.56	4.43	-0.13
(6.f)*	4.74	4.55	-0.19	(10.b)	4.58	4.53	-0.05
(6.g)	5.45	5.05	-0.40	(10.c)	4.58	4.56	-0.02
(6.h)	4.44	4.66	-0.22	(10.d)	4.05	4.48	-0.43
(6.i)*	4.03	4.13	0.10	(10.e)	4.40	4.18	-0.22
(7.a)	4.43	4.32	-0.11	(10.f)	4.92	4.74	-0.18
(7.b)*	4.58	4.51	-0.07	(10.g)	4.82	4.88	0.06
(7.c)	4.52	4.55	0.03	(10.h)	3.99	4.39	0.40
(7.d)	4.28	4.53	0.25	(10.i)	4.31	4.11	0.20
(7.e)	4.17	4.41	0.24				

*Testset compds, N. D: values not obtained.

Table 3. QSAR hypothesis Score

ID	Survival	Survival -inactive	Post-hoc	Site	Vector	Volume	Selectivity	# Matches	Energy	Activity	Inactive
ADRRRR.82	35.917	34.318	3.633	0.91	0.901	0.824	2.372	37	0	4.638	1.599
AARRRR.58	35.835	34.097	3.72	0.95	0.931	0.835	2.202	37	0	4.638	1.737
ADRRRR.80	35.791	34.164	3.516	0.85	0.862	0.804	2.362	37	0.01	4.42	1.627
ADRRRR.83	26.208	24.696	3.675	0.91	0.92	0.849	2.42	33	5.191	4.638	1.512
ADRRRR.84	26.206	24.702	3.67	0.91	0.909	0.847	2.422	33	5.169	4.602	1.504
AARRRR.60	26.105	24.492	3.744	0.95	0.947	0.85	2.247	33	5.191	4.638	1.613
√ADHRRR.1044	19.61	18.151	3.526	0.98	0.91	0.808	2.663	29	0.05	4.42	1.459

PHASE 3D-QSAR model is shown in Fig. 2d. The map showed a big gray colored region indicating that an increase in the hydrophobicity in this region is expected to improve the activity

of the *p*-hydroxy benzohydrazide-like molecules. A black color contour opposite to that of blue disfavors the placement of hydrophobic groups.

Table 4. PHASE 3D-QSAR statistical analysis

Pharmacophore	Model 1	Model 2	Model 3	Model 4
Hypothesis	ADRRRR	ADHRRR	AARRRR	ADRRRR
PLS statistics for QSAR model				
r^2	0.91	0.96	0.92	0.90
SD	0.11	0.13	0.13	0.13
F	72.1	54.1	28.2	20.1
P	$2.85e^{12}$	$4.98e^{13}$	$2.276e^{12}$	$1.96e^{13}$
No of PLS factors	4	4	4	4
External test set for prediction				
q^2	0.59	0.79	0.54	0.39
r_p	0.83	0.89	0.78	0.86
RMSE	0.37	0.38	0.29	0.27

SD: standard deviation, PLS: partial least square, $\sqrt{\quad}$ optimum model.

EXPERIMENTAL

Chemistry. General Procedures

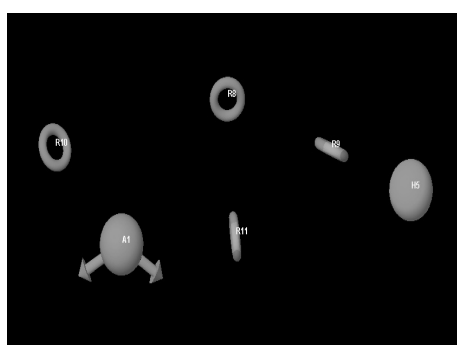
Chemicals were obtained from Fluka Chemical Co. (Germany). Melting points (m.p.) were detected with open capillaries using Thermo Precision Melting point cum Boiling point apparatus (C-PMB-2, Mumbai, India) and are uncorrected. IR spectra (KBr) were recorded on FTIR-8400s spectrophotometer (Shimadzu, Japan). ^1H NMR was obtained using a Varian

EM 390 Spectrophotometer (Shimadzu, Japan) using CDCl_3 . All chemical shift values were recorded as δ (ppm). The purity of compounds was controlled by thin layer chromatography (Merck, silica gel, $\text{HF}_{254-361}$, type 60, 0.25 mm, Darmstadt, Germany). The elementary analysis was performed at RTM Nagpur University, India. Elementary analyses for C, H, N were within $\pm 0.4\%$ of theoretical values.

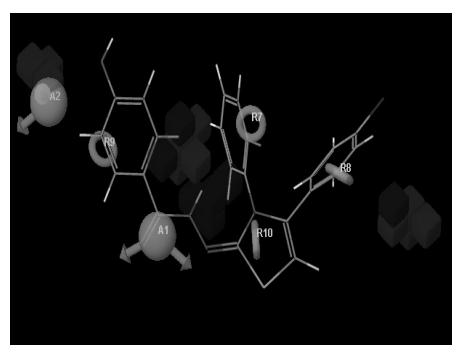
Synthesis of 2: A mixture of 1 (0.02 mol), 85% hydrazine hydrate (0.08 mol) was refluxed for 12 h. the excess solvent was removed under reduced pressure and the reaction mixture was cooled at $4 - 5^\circ$. The solid crystals separated were filtered washed with cold water dried and recrystallized from ethanol. To afford white product (2).

Yield: 1.32 g (80%). mp $170 - 171^\circ$ (ethanol:water), R_f : 0.62 (acetonitrile:methanol 1:1), IR (KBr): cm^{-1} 3351 (Alcohol O-H & C-O Stretching) 3013 (Ar-H Stretching), 1622 (C-O stretching), 1185, 1034 (alcohol O-H Stretching), 832 (benzene 1,4 disubstituted), $^1\text{H-NMR}$ (300 MHz, $\text{DMSO}-d_6$): δ 9.5 (s, 1H, CONH), 5.32 (s, 2H, NH_2). FABMS (m/z , 100%): 152 ($[\text{M}+2]$, 100%).

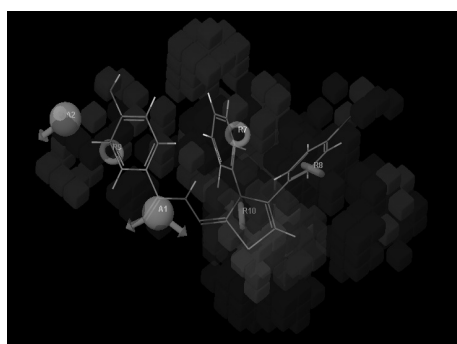
General procedure for synthesis of 3.a-3.i: To a solution of 2 (0.01 mol) in ethanol (50 mL), various aliphatic/aromatic isothiocyanates (0.01 mol) were added and the reaction mixture



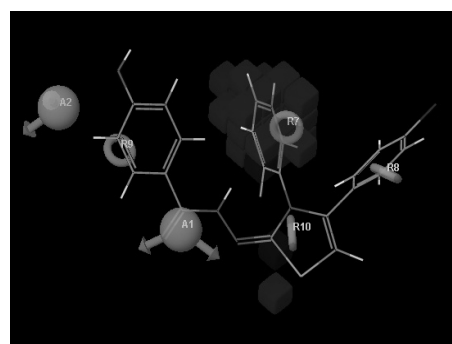
2a. Phase pharmacophore hypothesis (AHRRRR)



2b. Hydrogen bond donor of compd (12.f)



2c. Hydrophobic model of compd (12.f)



2d. Other effect of compd (12.f)

Fig. 2. Visual representation of atom-based PHASE QSAR.

was refluxed for 12 h. Excess solvent was removed under vacuum. The residue was washed with diethyl ether and recrystallized using methanol.

3.a. Yield: 1.0 g (70%), mp 225 - 226° (methanol), R_f 0.67 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3213, 3229 (NH stretching), 2986, 2990, ($\text{CH}(\text{CH}_3)_2$ stretching) 1732 (C=O stretching), 1315 (C=S stretching) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 1.2 (dd, 6H, isopropyl CH_3), 4.00 (m, 1H, isopropyl CH), 7.74 (s, 1H, CONH), 7.2-7.78 (m, 4H, Ar-H), 5.21 (s, 1H, -OH) FABMS (m/z , 100%): 253 ([M+2], 100%).

Anal. $\text{C}_{11}\text{H}_{15}\text{N}_3\text{O}_2\text{S}$; C, 52.13/52.15; H, 5.98/5.97; N, 16.59/16.59.

3.b. Yield: 1.1 g (74%), mp 168 - 169° (methanol), R_f 0.66 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3213, 3224 (NH stretching), 2981, 2986, 2990, ($\text{C}(\text{CH}_3)_3$ stretching) 1731 (C=O stretching), 1316 (C=S stretching) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 1.2 (dd, 9H, *t*-butyl CH_3), 4.72 (s, 2H, OCH_2), 7.73 (s, 1H, CONH), 6.8-7.78 (m, 4H, Ar-H), 5.3 (s, 1H, -OH) FABMS (m/z , 100%): 267 ([M+2], 100%).

Anal. $\text{C}_{12}\text{H}_{17}\text{N}_3\text{O}_2\text{S}$; C, 53.83/53.91; H, 6.41/6.41; N, 15.69/15.72.

3.c. Yield: 1.2 g (82%), mp 175 - 176° (methanol), R_f 0.66 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3217, 3232 (NH), 1738 (C=O stretching), 1313 (C=S stretching) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 5.35 (s, 1H, Ar-OH), 6.8-7.62 (m, 9H, Ar-H), 7.75 (s, 1H, CONH), FABMS (m/z , 100%): 287 ([M+2], 100%).

Anal. $\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_2\text{S}$; C, 58.53/58.20; H, 4.51/4.56; N, 14.59/14.62.

3.d. Yield: 0.8 g (56%), mp 189 - 190° (methanol), R_f 0.66 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3215, 3230 (NH), 1731 (C=O stretching), 1315 (C=S stretching) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 5.33 (s, 1H, Ar-OH), 6.88-7.82 (m, 8H, ArH), 7.78 (s, 1H, CONH), 10.40 (s, 1H, NH), 11.81 (s, 1H, NHAr), FABMS (m/z , 100%): 322 ([M+2], 100%).

Anal. $\text{C}_{14}\text{H}_{12}\text{N}_4\text{O}_4\text{S}$; C, 50.59/50.60; H, 3.64/3.64; N, 16.83/16.86.

3.e. Yield: 1.2 g (82%), mp 192 - 193° (methanol), R_f 0.7 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3217, 3232 (NH), 1738 (C=O stretching), 1313 (C=S stretching) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 5.35 (s, 1H, Ar-OH), 6.78-7.82 (m, 8H, ArH), 7.78 (s, 1H, CONH), 10.40 (s, 1H, NH), 11.81 (s, 1H, NHAr), FABMS (m/z , 100%): 305 ([M+2], 100%).

Anal. $\text{C}_{14}\text{H}_{12}\text{FN}_3\text{O}_2\text{S}$; C, 55.07/55.07; H, 3.94/3.96; N, 13.74/13.76.

3.f. Yield: 1.1 g (79%), mp 202 - 203° (methanol), R_f 0.62

(acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3212, 3235 (NH), 1734 (C=O stretching), 1317 (C=S stretching) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 5.22 (s, 1H, Ar-OH) 6.71-7.54 (m, 7H, ArH), 7.74 (s, 1H, CONH), 0.43 (s, 1H, NH), 11.82 (s, 1H, NHAr), FABMS (m/z , 100%): 356 ([M+2], 100%).

Anal. $\text{C}_{14}\text{H}_{11}\text{Cl}_2\text{N}_3\text{O}_2\text{S}$; C, 48.50/48.50; H, 2.81/2.80; N, 10.59/10.60.

3.g. Yield: 1.3 g (87%), mp 212 - 213° (methanol), R_f 0.56 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3217, 3232 (NH), 1738 (C=O stretching), 1313 (C=S stretching) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 5.35 (s, 1H, Ar-OH), 6.78-7.82 (m, 8H, ArH), 7.68 (s, 1H, CONH), 10.40 (s, 1H, NH), 11.81 (s, 1H, NHAr), FABMS (m/z , 100%): 323 ([M+2], 100%).

Anal. $\text{C}_{14}\text{H}_{11}\text{F}_2\text{N}_3\text{O}_2\text{S}$; C, 52.01/52.01; H, 3.39/3.43; N, 13.00/13.00

3.h. Yield: 1.0 g (69%), mp 198 - 199° (methanol), R_f 0.66 (acetonitrile:methanol 1:1), IR: 3217, 3232 (NH), 1738 (C=O stretching), 1313 (C=S stretching) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 5.30 (s, 1H, Ar-OH), 2.11 (d, 2H, CH_3) 6.78-7.82 (m, 7H, ArH), 7.66 (s, 1H, CONH), 10.44 (s, 1H, NH), 11.80 (s, 1H, NHAr), FABMS (m/z , 100%): 316 ([M+2], 100%).

Anal. $\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_2\text{S}$; C, 60.91/60.93; H, 5.39/5.43; N, 13.32/13.32.

3.i. Yield: (75%), mp 203 - 204° (methanol), R_f 0.57 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3219, 3230 (NH), 1732 (C=O stretching), 1310 (C=S stretching) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 5.31 (s, 1H, Ar-OH), 3.83 (d, 2H, OCH_3), 6.30-7.82 (m, 7H, ArH), 7.69 (s, 1H, CONH), 10.40 (s, 1H, NH), 11.80 (s, 1H, NHAr), FABMS (m/z , 100%): 347 ([M++2], 100%), anal. found C(55.31%) H(4.90%) N(12.08%): Calculated C(55.32%) H(4.93%) N(12.10%).

General procedure for synthesis of compound 4.a-4.i: A mixture of the thiosemicarbazide (0.01 mol), chloroacetic acid (0.01 mol) and sodium acetate (0.2 mol) in ethanol (60 mL) was refluxed for 10 h. The mixture was cooled, diluted with enough water to develop turbidity and left overnight to obtain the product. The product was filtered, dried and recrystallized using aqueous ethanol.

4.a. Yield: 2.21 g (86%), mp 225 - 226° (methanol), R_f 0.55 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3212 (NH stretching), 2986, 2990, ($\text{CH}(\text{CH}_3)_2$ stretching) 1732 (C=O stretching), 1315 (C=S stretching) cm^{-1} ; $^1\text{H NMR}$ (CDCl_3): δ 1.12 (dd, 6H, isopropyl CH_3), 3.96 (m, 1H, isopropyl CH), 5.31 (s, 1H, Ar-OH), 3.01 (s, 2H, thiazolidine CH_2), 7.74 (s, 1H, CONH), FABMS (m/z , 100%): 293 ([M+2], 100%).

Anal. $\text{C}_{13}\text{H}_{15}\text{N}_3\text{O}_3\text{S}$; C, 53.21/53.23; H, 5.15/5.15; N, 14.30/

14.32.

4.b. Yield: 2.2 g (85%), mp 206 - 207^o (methanol), *R_f* 0.59 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 2982, 2987, 2989, (C(CH₃)₃ stretching) 1732 (C=O stretching), 3212 (NH stretching) cm^{-1} ; ¹H NMR (CDCl₃): δ 1.2 (dd, 9H, t-butyl CH₃), 3.01 (s, 2H, thiazolidine CH₂), 4.72 (s, 1H, NH), 5.35 (s, 1H, Ar-OH), 7.73 (s, 1H, CONH) FABMS (*m/z*, 100%): 307 ([M+2], 100%).
Anal. C₁₄H₁₇N₃O₃S; C, 53.91/53.91; H, 6.35/6.41; N, 15.62/15.72.

4.c. Yield: 2.1 g (84%), mp 256 - 257^o (methanol), *R_f* 0.66 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 1732 (C=O stretching), 3218 (NH stretching) cm^{-1} ; ¹H NMR (CDCl₃): δ 3.04 (s, 2H, thiazolidine CH₂), 4.72 (s, 1H, NH), 5.35 (s, 1H, Ar-OH), 7.73 (s, 1H, CONH), 6.38-7.85 (m, 9H, Ar-H), FABMS (*m/z*, 100%): 327 ([M+2], 100%).
Anal. C₁₆H₁₃N₃O₃S; C, 58.51/58.52; H, 4.55/4.56; N, 14.52/14.62.

4.d. Yield: 1.6 g (52%), mp 241 - 242^o (methanol), *R_f* 0.56 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3214 (NH stretching), 1731 (C=O stretching), 1555 (NO₂ stretching); ¹H NMR (CDCl₃): δ 3.98 (s, 2H, thiazolidine CH₂), 4.60 (s, 1H, NH), 5.23 (s, 1H, Ar-OH), 7.70 (s, 1H, CONH), 6.38-7.85 (m, 8H, Ar-H), FABMS (*m/z*, 100%): 372 ([M+2], 100%).
Anal. C₁₆H₁₂N₄O₅S; C, 50.51/50.60; H, 3.55/3.64; N, 16.82/16.86.

4.e. Yield: 2.7 g (89%), mp 223 - 224^o (methanol), *R_f* 0.7 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3220 (NH stretching), 1792 (C=O stretching), 1045 (C-F stretching); ¹H NMR (CDCl₃): δ 3.76 (s, 2H, thiazolidine CH₂), 4.36 (s, 1H, NH), 5.3 (s, 1H, Ar-OH), 8.04 (s, 1H, CONH), 6.38-7.85 (m, 8H, Ar-H), FABMS (*m/z*, 100%): 372 ([M+2], 100%).
Anal. C₁₆H₁₂FN₃O₃S; C, 55.05/55.07; H, 3.95/3.96; N, 13.78/13.76.

4.f. Yield: 2.6 g (75%), mp 215 - 216^o (methanol), *R_f* 0.56 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3220 (NH stretching), 1743 (C=O stretching); ¹H NMR (CDCl₃): δ 3.76 (s, 2H, thiazolidine CH₂), 4.30 (s, 1H, NH), 5.28 (s, 1H, Ar-OH), 7.82 (s, 1H, CONH), 6.38-7.85 (m, 7H, Ar-H), FABMS (*m/z*, 100%): 396 ([M+2], 100%).
Anal. C₁₆H₁₁Cl₂N₃O₃S; C, 47.21/47.20; H, 3.11/3.11; N, 11.76/11.80.

4.g. Yield: 2.4 g (78%), mp 219 - 220^o (methanol), *R_f* 0.56 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3280 (NH stretching), 1773 (C=O stretching); ¹H NMR (CDCl₃): δ 3.06 (s, 2H,

thiazolidine CH₂), 4.36 (s, 1H, NH), 5.23 (s, 1H, Ar-OH), 7.82 (s, 1H, CONH), 6.38-7.85 (m, 7H, Ar-H), FABMS (*m/z*, 100%): 363 ([M+2], 100%).

Anal. C₁₆H₁₁F₂N₃O₃S; C, 52.01/52.01; H, 3.41/3.43; N, 13.00/13.00.

4.h. Yield: 2.6 g (87%), mp 217 - 218^o (methanol), *R_f* 0.61 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3280 (NH stretching), 1773 (C=O stretching); ¹H NMR (CDCl₃): δ 1.47 (d, 3H, CH₃), 3.16 (s, 2H, thiazolidine CH₂), 4.26 (s, 1H, NH), 5.34 (s, 1H, Ar-OH), 7.62 (s, 1H, CONH), 6.48-7.85 (m, 7H, Ar-H), FABMS (*m/z*, 100%): 355 ([M+2], 100%).

Anal. C₁₈H₁₇N₃O₃S; C, 60.91/60.93; H, 5.43/5.43; N, 13.30/13.32.

4.i. Yield: 2.6 g (84%), mp 203 - 204^o (methanol), *R_f* 0.61 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3280 (NH stretching), 1773 (C=O stretching); ¹H NMR (CDCl₃): δ 3.73 (s, 6H, OCH₃), 3.76 (s, 2H, thiazolidine CH₂), 4.16 (s, 1H, NH), 5.41 (s, 1H, Ar-OH), 7.82 (s, 1H, CONH), 6.48-7.85 (m, 7H, Ar-H), FABMS (*m/z*, 100%): 387 ([M+2], 100%).

Anal. C₁₈H₁₇N₃O₅S; C, 55.31/55.32; H, 4.90/4.93; N, 12.06/12.10.

General Procedure for synthesis of compounds from (5.a-5.i) to (10.a-10.i): Specific examples presented below illustrate general synthetic procedures.

The mixture of the thiosemicarbazide (0.01 mol) (**3.a-3.i**) appropriate phenacyl bromide (0.01 mol) and sodium acetate (0.2 mol) in ethanol (50 mL) was refluxed for 7 h. The mixture was cooled, diluted with enough water to develop turbidity and left overnight to obtain the product. The product was filtered, dried and recrystallized using aqueous ethanol.

5.a. Yield: 1.3 g (53%), mp 156 - 157^o (Ethanol: Water), *R_f* 0.54 (acetonitrile: methanol 1:1), IR (KBr) cm^{-1} : 1732 (C=O stretching), 1573, 1488, 1056 (thiazoline), 3002 (ArH stretching); ¹H NMR (CDCl₃): δ 1.23 (dd, 6H, isopropyl CH₃), 4.03 (m, 1H, isopropyl CH), 5.22 (s, 1H, Ar-OH), 6.06 (s, 1H, thiazoline), 7.08-7.76 (m, 4H, ArH), 7.79 (s, 1H, CONH), 6.48-8.05 (m, 9H, Ar-H), FABMS (*m/z*, 100%): 353 ([M+2], 100%).

Anal. C₁₉H₁₉N₃O₂S; C, 64.56/64.57; H, 5.43/5.42; N, 11.90/11.89.

5.b. Yield: 1.6 g (63%), mp 161 - 162^o (Ethanol: Water), *R_f* 0.67 (acetonitrile:methanol 1:1), IR (KBr) cm^{-1} : 3226 (NH), 1732 (C=O stretching), 1575, 1481, 1053 (thiazoline), 3002 (ArH stretching); ¹H NMR (CDCl₃): δ 0.94 (t, 3H, butyl CH₃), 1.39 (m, 2H, butyl CH₂), 1.53 (m, 2H, butyl CH₂), 3.62 (m, 2H, butyl CH₂), 5.31 (s, 1H, Ar-OH), 5.99 (s, 1H, thiazoline),

7.16-7.68 (m, 9H, Ar-H), 7.77 (s, 1H, CONH), FABMS (*m/z*, 100%): 367 ([M+2], 100%).

Anal. C₂₀H₂₁N₃O₂S; C, 65.35/65.37; H, 5.73/5.76; N, 11.46/11.44.

5.e. Yield: 1.8 g (57%), mp 192 - 193° (Ethanol: Water), R_f 0.59 (acetonitrile:methanol 1:1), IR (KBr) cm⁻¹: 3229 (NH), 1742 (C=O stretching), 1545, 1472, 1011 (thiazoline), 3014 (ArH stretching); ¹H NMR (CDCl₃): δ 5.18 (s, 1H, Ar-OH), 5.91 (s, 1H, thiazoline), 7.05-7.69 (m, 12H, ArH), 7.77 (s, 1H, CONH), FABMS (*m/z*, 100%): 405 ([M+2], 100%).

Anal. C₂₂H₁₆FN₃O₂S; C, 65.12/65.17; H, 3.93/3.98; N, 10.35/10.35.

5.h. Yield: 2.1 g (69%), mp 201 - 202° (Ethanol: Water), R_f 0.52 (acetonitrile:methanol 1:1), IR (KBr) cm⁻¹: 3214 (NH), 1723 (C=O stretching), 1561, 1484, 1055 (thiazoline), 3013 (ArH stretching); ¹H NMR (CDCl₃): δ 1.28 (t, 3H, CH₂CH₃), 3.65 (q, 2H, CH₂CH₃), 5.33 (s, 1H, Ar-OH), 6.17 (s, 1H, thiazoline), 7.03-7.67 (m, 11H, ArH), 7.82 (s, 1H, CONH), FABMS (*m/z*, 100%): 415 ([M+2], 100%).

Anal. C₂₄H₂₁N₃O₂S; C, 67.36/69.37; H, 5.07/5.09; N, 10.10/10.11.

5.i. Yield: 2.6 g (76%), mp 197 - 198° (Ethanol: Water), R_f 0.62 (acetonitrile:methanol 1:1), IR (KBr) cm⁻¹: 3225 (NH), 1733 (C=O stretching), 1578, 1482, 1052 (thiazoline), 3005 (ArH stretching); ¹H NMR (CDCl₃): δ 5.23 (s, 1H, Ar-OH), 6.07 (s, 1H, thiazoline), 7.09-7.72 (m, 11H, ArH), 7.78 (s, 1H, CONH), FABMS (*m/z*, 100%): 456 ([M+2], 100%).

Anal. C₂₂H₁₅Cl₂N₃O₂S; C, 57.92/57.90; H, 3.30/3.31; N, 9.19/9.21.

6.e. Yield: 2.1 g (72%), mp 170 - 171° (Ethanol: Water), R_f 0.53 (acetonitrile:methanol 1:1), IR (KBr) cm⁻¹: 3223 (NH), 1740 (C=O stretching), 1570, 1480, 1052 (thiazoline), 3006 (ArH stretching); ¹H NMR (CDCl₃): δ 5.41 (s, 1H, Ar-OH), 6.01 (s, 1H, thiazoline), 7.08-7.61 (m, 12H, ArH), 7.79 (s, 1H, CONH), FABMS (*m/z*, 100%): 440 ([M+2], 100%).

Anal. C₂₂H₁₅ClFN₃O₂S; C, 60.06/60.07; H, 3.45/3.44; N, 9.55/9.55.

6.f. Yield: 2.6 g (76%), mp 194 - 195° (Ethanol: Water), R_f 0.72 (acetonitrile:methanol 1:1), IR (KBr) cm⁻¹: 3224 (NH), 1739 (C=O stretching), 1573, 1485, 1056 (thiazoline), 3003 (ArH stretching); ¹H NMR (CDCl₃): δ 5.33 (s, 1H, Ar-OH), 5.97 (s, 1H, thiazoline), 7.09-8.01 (m, 11H, ArH), 7.76 (s, 1H, CONH), FABMS (*m/z*, 100%): 490 ([M+2], 100%).

Anal. C₂₂H₁₄Cl₃N₃O₂S; C, 53.86/53.84; H, 2.87/2.88; N, 8.55/8.56.

6.g. Yield: 2.2 g (70%), mp 205 - 206° (Ethanol: Water), R_f

0.56 (acetonitrile:methanol 1:1), IR (KBr) cm⁻¹: 3220 (NH), 1740 (C=O stretching), 1563, 1481, 1060, (thiazoline), 3013 (ArH stretching); ¹H NMR (CDCl₃): δ 2.33 (s, 1H, Ar-OH), 5.87 (s, 1H, thiazoline), 7.09-8.01 (m, 11H, ArH), 7.81 (s, 1H, CONH), FABMS (*m/z*, 100%): 458 ([M+2], 100%).

Anal. C₂₂H₁₄ClF₂N₃O₂S; C, 57.72/57.71; H, 3.07/3.08; N, 9.18/9.18.

6.h. Yield: 1.95 g (63%), mp 198 - 199° (Ethanol: Water), R_f 0.56 (acetonitrile:methanol 1:1), IR (KBr) cm⁻¹: 3220 (NH), 1740 (C=O stretching), 1568, 1482, 1064, (thiazoline), 3021 (ArH stretching); ¹H NMR (CDCl₃): δ 2.34 (s, 6H, CH₃), 2.33 (s, 1H, Ar-OH), 5.87 (s, 1H, thiazoline), 6.76-7.79 (m, 11H, ArH), 7.80 (s, 1H, CONH), FABMS (*m/z*, 100%): 449 ([M+2], 100%).

Anal. C₂₄H₂₀ClN₃O₂S; C, 64.06/64.06; H, 4.47/4.48; N, 9.34/9.34.

7.h. Yield: 2.0 g (67%), mp 218 - 219° (Ethanol: Water), R_f 0.56 (acetonitrile:methanol 1:1), IR (KBr) cm⁻¹: 3222 (NH), 1743 (C=O stretching), 1565, 1483, 1066, (thiazoline), 3021 (ArH stretching); ¹H NMR (CDCl₃): δ 2.14 (s, 6H, CH₃), 5.30 (s, 1H, Ar-OH), 5.90 (s, 1H, thiazoline), 6.96-7.81 (m, 11H, ArH), 7.80 (s, 1H, CONH), FABMS (*m/z*, 100%): 494 ([M+2], 100%).

Anal. C₂₄H₂₀BrN₃O₂S; C, 58.32/58.30; H, 4.07/4.08; N, 8.52/8.50.

7.i. Yield: 2.0 g (62%), mp 245 - 246° (Ethanol: Water), R_f 0.65 (acetonitrile:methanol 1:1), IR (KBr) cm⁻¹: 3234 (NH), 1744 (C=O stretching), 1573, 1481, 1053 (thiazoline), 3009 (ArH stretching); ¹H NMR (CDCl₃): δ 3.86 (s, 1H, OCH₃-C₆H₄), 5.32 (s, 1H, Ar-OH), 6.77 (s, 1H, thiazoline), 6.89-7.86 (m, 11H, ArH), 7.76 (s, 1H, CONH), FABMS (*m/z*, 100%): 524 ([M+2], 100%).

Anal. C₂₄H₂₀BrN₃O₄S; C, 54.76/54.76; H, 3.82/3.83; N, 7.89/7.98.

8.g. Yield: 2.4 g (78%), mp 189 - 190° (Ethanol: Water), R_f 0.62 (acetonitrile:methanol 1:1), IR (KBr) cm⁻¹: 3218 (NH), 1741 (C=O stretching), 1565, 1479, 1058, (thiazoline), 3003 (ArH stretching); ¹H NMR (CDCl₃): δ 2.33 (s, 1H, Ar-CH₃), 5.31 (s, 1H, Ar-OH), 6.92 (s, 1H, thiazoline), 6.72-7.75 (m, 11H, Ar-H), 7.01 (s, 1H, CONH), FABMS (*m/z*, 100%): 438 ([M+2], 100%).

Anal. C₂₃H₁₇F₂N₃O₂S; C, 63.15/63.15; H, 3.91/3.92; N, 9.60/9.61.

8.h. Yield: 1.8 g (61%), mp 221 - 222° (Ethanol: Water), R_f 0.63 (acetonitrile:methanol 1:1), IR (KBr) cm⁻¹: 3217 (NH), 1740 (C=O stretching), 1562, 1481, 1061, (thiazoline), 3018

(ArH stretching); $^1\text{H NMR}$ (CDCl_3): δ 2.32 (s, 1H, Ar- CH_3), 2.22 (s, 6H, CH_3), 5.30 (s, 1H, Ar-OH), 6.22 (s, 1H, thiazoline), 6.86-7.81 (m, 11H, ArH), 7.80 (s, 1H, CONH), FABMS (m/z , 100%): 429 ($[\text{M}+2]$, 100%).

Anal. $\text{C}_{25}\text{H}_{23}\text{N}_3\text{O}_2\text{S}$; C, 68.90/69.91; H, 5.41/5.40; N, 9.77/9.78.

8.i. Yield: 2.2 g (67%), mp 257 - 258 $^\circ$ (Ethanol: Water), R_f 0.55 (acetonitrile: methanol 1:1), IR (KBr) cm^{-1} : 3234 (NH), 1744 (C=O stretching), 1573, 1481, 1053 (thiazoline), 3009 (ArH stretching); $^1\text{H NMR}$ (CDCl_3): δ 2.33 (s, 1H, Ar- CH_3), 3.71 (s, 1H, $\text{OCH}_3\text{-C}_6\text{H}_4$), 5.31 (s, 1H, Ar-OH), 6.56 (s, 1H, thiazoline), 6.89-7.86 (m, 11H, ArH), 7.81 (s, 1H, CONH), FABMS (m/z , 100%): 462 ($[\text{M}+2]$, 100%).

Anal. $\text{C}_{25}\text{H}_{23}\text{N}_3\text{O}_4\text{S}$; C, 65.06/65.06; H, 5.01/5.02; N, 9.11/9.10.

9.h. Yield: 1.8 g (62%), mp 222 - 223 $^\circ$ (Ethanol: Water), R_f 0.63 (acetonitrile: methanol 1:1), IR (KBr) cm^{-1} : 3217 (NH), 1740 (C=O stretching), 1562, 1481, 1061, (thiazoline), 3018 (ArH stretching); $^1\text{H NMR}$ (CDCl_3): δ 2.12 (s, 6H, CH_3), 5.22 (s, 1H, Ar-OH), 6.95 (s, 1H, thiazoline), 6.67-7.81 (m, 11H, ArH), 7.77 (s, 1H, CONH), FABMS (m/z , 100%): 431 ($[\text{M}+2]$, 100%).

Anal. $\text{C}_{24}\text{H}_{21}\text{N}_3\text{O}_3\text{S}$; C, 66.80/66.80; H, 4.90/4.91; N, 9.72/9.74.

9.i. Yield: 2.2 g (75%), mp 210 - 211 $^\circ$ (Ethanol: Water), R_f 0.55 (acetonitrile: methanol 1:1), IR (KBr) cm^{-1} : 3234 (NH), 1744 (C=O stretching), 1573, 1481, 1053 (thiazoline), 3009 (ArH stretching); $^1\text{H NMR}$ (CDCl_3): δ 3.76 (s, 1H, $\text{OCH}_3\text{-C}_6\text{H}_4$), 5.29 (s, 1H, Ar-OH), 6.65 (s, 1H, thiazoline), 6.91-7.85 (m, 11H, ArH), 7.64 (s, 1H, CONH), FABMS (m/z , 100%): 464 ($[\text{M}+2]$, 100%).

Anal. $\text{C}_{24}\text{H}_{21}\text{N}_3\text{O}_5\text{S}$; C, 62.17/62.19; H, 4.51/4.57; N, 8.98/9.07.

10.d. Yield: 1.4 g (45%), mp 209 - 210 $^\circ$ (Ethanol: Water), R_f 0.63 (acetonitrile: methanol 1:1), IR (KBr) cm^{-1} : 3218 (NH), 1556 (Ar- NO_2 stretching), 1731 (C=O stretching), 1566, 1473, 1054 (thiazoline), 3009 (ArH stretching); $^1\text{H NMR}$ (CDCl_3): δ 3.41 (s, $\text{OCH}_3\text{-C}_6\text{H}_4$), 5.29 (s, 1H, Ar-OH), 7.95 (s, 1H, thiazoline), 6.75-7.76 (m, 12H, Ar-H), 7.40 (s, 1H, CONH), FABMS (m/z , 100%): 463 ($[\text{M}+2]$, 100%).

Anal. $\text{C}_{23}\text{H}_{18}\text{N}_4\text{O}_5\text{S}$; C, 59.74/59.67; H, 3.92/3.86; N, 11.88/12.11.

10.f. Yield: 2.3 g (66%), mp 208 - 209 $^\circ$ (Ethanol: Water), R_f 0.67 (acetonitrile: methanol 1:1), IR (KBr) cm^{-1} : 790 (Ar-Cl stretching), 3245 (NH), 1739 (C=O stretching), 1543, 1465, 1062, (thiazoline), 3007 (ArH stretching); $^1\text{H NMR}$ (CDCl_3):

δ 3.24 (s, 1H, $\text{OCH}_3\text{-C}_6\text{H}_5$), 5.31 (s, 1H, Ar-OH), 6.93 (s, 1H, thiazoline), 6.88-8.01 (m, 11H, ArH), 7.16 (s, 1H, CONH), FABMS (m/z , 100%): 476 ($[\text{M}+2]$, 100%).

Anal. $\text{C}_{23}\text{H}_{17}\text{Cl}_2\text{N}_3\text{O}_3\text{S}$; C, 56.82/56.80; H, 3.53/3.52; N, 8.63/8.64.

10.g. Yield: 2.0 g (65%), mp 212 - 213 $^\circ$ (Ethanol: Water), R_f 0.57 (acetonitrile: methanol 1:1), IR (KBr) cm^{-1} : 1315 (Ar-F stretching), 3225 (NH), 1741 (C=O stretching), 1554, 1470, 1048, (thiazoline), 3006 (ArH stretching); $^1\text{H NMR}$ (CDCl_3): δ 3.25 (s, 1H, $\text{OCH}_3\text{-C}_6\text{H}_5$), 5.27 (s, 1H, Ar-OH), 6.90 (s, 1H, thiazoline), 6.70-7.88 (m, 11H, Ar-H), 7.06 (s, 1H, CONH), FABMS (m/z , 100%): 454 ($[\text{M}+2]$, 100%).

Anal. $\text{C}_{23}\text{H}_{17}\text{F}_2\text{N}_3\text{O}_3\text{S}$; C, 60.91/60.92; H, 3.56/3.78; N, 9.16/9.27.

10.h. Yield: 2.3 g (76%), mp 222 - 223 $^\circ$ (Ethanol: Water), R_f 0.68 (acetonitrile: methanol 1:1), IR (KBr) cm^{-1} : 3221 (NH), 1732 (C=O stretching), 1554, 1475, 1057, (thiazoline), 3001 (ArH stretching); $^1\text{H NMR}$ (CDCl_3): δ 3.11 (s, 1H, $\text{OCH}_3\text{-C}_6\text{H}_5$), 2.25 (s, 6H, CH_3), 5.28 (s, 1H, Ar-OH), 7.01 (s, 1H, thiazoline), 6.67-7.81 (m, 11H, ArH), 7.64 (s, 1H, CONH), FABMS (m/z , 100%): 445 ($[\text{M}+2]$, 100%).

Anal. $\text{C}_{25}\text{H}_{23}\text{N}_3\text{O}_3\text{S}$; C, 67.72/67.40; H, 5.22/5.20; N, 9.42/9.43.

10.i. Yield: 2.1 g (63%), mp 208 - 209 $^\circ$ (Ethanol: Water), R_f 0.60 (acetonitrile: methanol 1:1), IR (KBr) cm^{-1} : 3231 (NH), 1737 (C=O stretching), 1554, 1470, 1049 (thiazoline), 3010 (ArH stretching); $^1\text{H NMR}$ (CDCl_3): δ 3.26 (s, 1H, $\text{OCH}_3\text{-C}_6\text{H}_4$), 5.35 (s, 1H, Ar-OH), 6.78 (s, 1H, thiazoline), 6.88-7.85 (m, 11H, ArH), 7.58 (s, 1H, CONH), FABMS (m/z , 100%): 477 ($[\text{M}+2]$, 100%).

Anal. $\text{C}_{25}\text{H}_{23}\text{N}_3\text{O}_5\text{S}$; C, 62.87/62.88; H, 4.86/4.85; N, 8.78/8.80.

Antibacterial activity

The inoculum was prepared with fresh cultures of bacterial strains, cultured on plate count agar (PCA-Merck, Germany) for 18 h at 35 $^\circ\text{C}$. The density of the inoculum was adjusted according to Mac Farland n.1 scale.¹⁶ The minimal inhibitory concentration, MIC, was determined by the broth twofold macrodilution method in Tryptic Soy Broth (TSB-Difco Laboratories, Detroit, USA), using the serial dilution tests¹⁷⁻²⁰ in two sequential steps against standard (ATCC 25923) and multidrug-resistant (3SP/R33)¹⁹ *S. aureus* strains. Initially, stock solutions of $\text{N}^{\text{7}}\text{-}[(3\text{-substituted-4-oxo-1,3-thiazolidin-2-ylidene})\text{-4-hydroxybenzohydrazide}]$ and $\text{N}^{\text{7}}\text{-}[(3,4\text{-disubstituted})\text{-1,3-thiazolidin-2-ylidene})\text{-4-hydroxybenzohydrazide}]$ were prepared in dimethylsulfoxide (DMSO-Merck, Germany) and then diluted in culture medium, TSB. The tubes were inoculated with a stan-

standardized number of microorganisms and incubated at 35 °C for 18 h, after which the tubes were examined for visible signs of bacterial growth. MIC was defined as the lowest concentration of a compound that completely inhibited the bacterial growth. All experiments were performed in quadruplicate.

3D-QSAR study

We performed 3D-QSAR analysis on the previously synthesized and evaluated derivatives of 3D-QSAR study of *N'*-[-(3-substituted-alkyl/aryl)-4-(Substituted Aryl)-1,3-thiazolidin-2-ylidene]-4-hydroxybenzohydrazide and 4-hydroxy-*N'*-3-Substituted-4-oxo-1,3-thiazolidin-2-ylidene]benzohydrazide derivatives (Table 2) against as a antibacterial agents.

The software use for 3D-QSAR study is Schrödinger PHASE Module Workstation used are raster systems in which a computer with Linux as operating systems, 180 giga bite space storage facility Intel Pentium IV as a processor and integrated with graphical display. PHASE module works as a following five stapes as:

1. Preparing ligands: The 3-D conversion and minimization was performed using LigPrep (MMFF force field) incorporated in PHASE. A maximum of 100 conformers were generated per structure using a preprocess minimization of 100 steps and post process minimization of 50 steps. Each minimized conformer was filtered through a relative energy window of 11.4 kCal/mol (50 kJ/mol) and a minimum atom deviation of 2.00 Å.

2. Creating pharmacophore sites: The second step in developing a pharmacophore model is to use a set of pharmacophore features to create sites for all the ligands.

3. Finding a common pharmacophore: Active and inactive thresholds of pIC_{50} 4.1 and 4.5, respectively, were applied to the training set for developing the common pharmacophore hypotheses. After applying default feature definitions to each ligand, common pharmacophores containing six sites were generated using a terminal box size of 1 Å, and with requirement that all actives should match.

4. Scoring Hypotheses: In the score hypotheses step, common pharmacophores are examined, and a scoring procedure is applied to identify the pharmacophore from each surviving *n*-dimensional box that yields the best alignment of the active set ligands. The results are summarized in Table 3.

5. Building QSAR model: PHASE provides the means to build QSAR models using the activities of the ligands that match a given hypothesis. PHASE QSAR models are based on PLS regression, applied to a large set of binary valued variables. The independent variables in the QSAR model are derived from a

regular grid of cubic volume elements that span the space occupied by the training set ligands. Each ligand is represented by a set of bit values (0 or 1) that indicate which volume elements are occupied by a Vander Waals surface model of the ligand. As the compd (12.f) showed good activity, the common pharmacophore were generated for the best PHASE hypothesis with this compd (Table 3 and Fig. 2a). PHASE 3D plots of crucial pharmacophore region based on hypothesis generated were displayed with compd (12.f). Positive coefficient favored areas (contributing for increase in activity) were represented by blue cubes. Negative coefficient favored areas (contributing for decrease in activity) were represented by red cubes and are shown in Fig. 2b. to Fig. 2d. The summary of PHASE 3D-QSAR statistical analysis is given in Table 4.

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